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Characterization of Viremia at Different Stages of Varicella-Zoster Virus Infection

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Varicella-zoster virus (VZV) viremia at different stages of infection was characterized. Different approaches were used, polymerase chain reaction (PCR), isothermal transcription based nucleic acid amplification (NASBA), and immunofluorescence to describe and quantitate viral infection of peripheral blood mononuclear cells (PBMC). In patients with acute varicella 200 to 5,000 copies of the viral genome in every 150,000 PBMC were found with quantitative competitive PCR (QCPCR). With NASBA, viral transcriptional activity was detected in these cells. RNA transcribed from the immediate early gene IE 63 as well as from the late gene 68 were found, indicating a productive infection. Glycoprotein gE specific immunofluorescence visualized by confocal laser scanning microscopy revealed that only 1 in 10,000 to 100,000 PBMC was infected. T and B lymphocytes as well as monocytes expressed viral protein on their surface. Similar results were obtained with PBMC from immunocompetent zoster patients. In some cases a transient viremia was found shortly after the onset of rash, although the viral load seemed to be lower than in patients with varicella. Examination of blood samples from 16 persons with postherpetic neuralgia (PHN) signs of viral replication in PBMC were not detected. In conclusion, the data suggest that VZV viremia is a frequent event in patients with varicella and zoster, but not in those with postherpetic neuralgia. Moreover, the results indicated that subclinical reactivations occur both in immunocompromised and immunocompetent individuals. ***J. Med. Virol. 56:91–98, 1998.***

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KEY WORDS: PBMC-associated viremia; PCR; NASBA; immunofluorescence

INTRODUCTION

Primary infection with varicella-zoster virus (VZV) results in chickenpox (varicella). Recent data suggest that the virus is spread throughout the body a few days before and after the onset of the rash by cell-associated viremia, as VZV can be detected in peripheral blood mononuclear cells (PBMC) by cocultivation, in situ hybridization and polymerase chain reaction (PCR) [Arvin, 1996]. The virus establishes a life-long latent infection of sensory ganglia. Reactivation from latency correlates with a decline in VZV-specific cellular immunity, because the clinical manifestation of reactivation, herpes zoster, is predominantly seen in older and immunocompromised individuals. The disease is characterized by pain and a vesicular rash, which are generally limited to the dermatome innervated by a single sensory ganglion, but occasionally one or few extradermatomal lesions occur.

Postherpetic neuralgia is a frequent complication of zoster, particularly in elderly patients. It is defined as pain that persists for more than a month after the onset of the rash. Whether the pathogenesis of postherpetic neuralgia is due to continuous viral replication or neuronal cell damage is controversial [Vafai et al., 1988; Kost and Straus, 1996].

Various experimental approaches were used to detect VZV-specific nucleic acids and proteins. With PCR viral DNA was sought in PBMC taken from patients in different stages of VZV infection. To examine whether infected cells contained the complete viral genome, primers were chosen to amplify the late gene 14, the early gene 29, and the immediate early gene 63, which are located in different regions. NASBA (isothermal transcription based nucleic acid amplification) as well as immunofluorescence were performed to investigate productive infection of this cell type. NASBA was used to detect RNA transcribed from gene 63 and from gene 68. The latter codes for the glycoprotein gE, the most abundant viral protein on the surface of infected cells. Thus, this glycoprotein was also chosen as a target in immunofluorescence studies.

The aim of the study was to characterize the viremic

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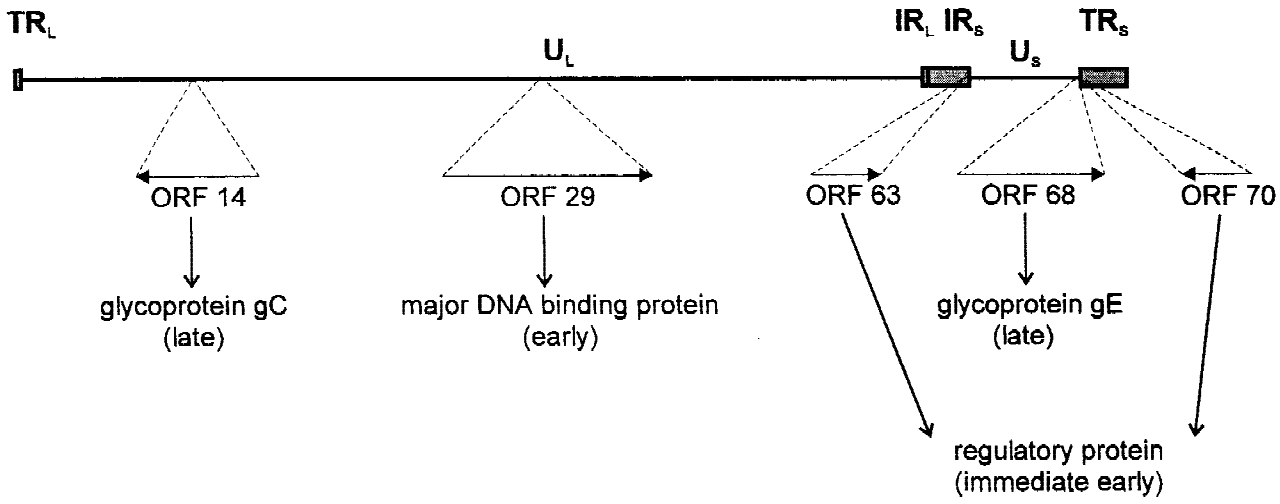

Fig. 1. Schematic representation of the VZV genome and open reading frames (ORFs) used for PCR and NASBA. Inverted repeats (TR_L/IR_L and IR_S/TR_S) flanking the unique regions U_L and U_S are shown as rectangles.

phase in patients with acute varicella or zoster as well as to determine whether VZV-infected PBMC can be found in patients suffering from postherpetic neuralgia. Furthermore, VZV specific sequences were sought in immunocompetent and immunocompromised individuals without any signs of VZV-caused illness. The detection of viral DNA, RNA; and proteins should allow the distinction between nonproductive, latent, and productive infections of PBMC.

MATERIALS AND METHODS

Study Population

Blood specimens were obtained after informed consent from a variety of patients with different degrees of VZV infection: 21 patients with typical clinical features of varicella, 71 patients with zoster, and 16 persons with postherpetic neuralgia. Samples were also collected from 149 VZV-seropositive adults without any VZV-induced symptoms, 99 were immunocompetent (21 <60 years old, 78 >60 years old), the other 50 were immunocompromised either because of chemotherapy or HIV-1-infection, with <500 CD4 cells/mm^3. Specimens were also drawn from 14 individuals without antibodies to VZV. Serological data (IgG and IgM) were determined for each specimen by ELISA (Virotech, Germany).

Cells and Viruses

PBMC were recovered by ficoll-hypaque density gradient centrifugation. Viruses for positive and negative controls (VZV strains Ellen, Oka and Webster, HSV-1 strain F) were grown on human embryonic lung fibroblasts or human skin fibroblasts. Nucleic acids from infected or uninfected cells were extracted in the same manner as from PBMC.

Target Sequences, Primers, and Oligonucleotide Probes

Location of the open reading frames (ORFs) used for detection by PCR and NASBA are shown in Figure 1. The late gene 14 is located near the 5′-end of the VZV genome in the unique long region and coding for the glycoprotein gC. The early gene 29 is located in the middle of the unique long region and codes for the major DNA-binding protein. The immediate early gene 63 is found in the inverted repeat sequences flanking the unique short region is identical with gene 70 and codes for the regulatory protein IE63. Sequences of oligonucleotides were chosen from the published VZV sequence [Davison and Scott, 1986]. The software program Primer Select (DNAStar, U.K.) was utilized to optimize the design of PCR and NASBA primers. Primers, probe, and protocol for the ORF-63 PCR were modified from those published by Dlugosch et al. [1991]. Primers and probes for PCR (ORF 14, 29, and 63) and NASBA (ORF 63 and 68) are listed in Table I.

Plasmids

VZV DNA derived from pAT 153 KpnI sf, pAT 153 SstI f (generously provided by Dr. A.J. Davison, University of Glasgow, UK) and pGem EcoRI b. Restriction endonuclease fragments of VZV DNA corresponding to ORF 14, 29, 63, and 68 were subcloned into pT7/T3-α19 and pSport (Life Technologies, Germany).

Sample Preparation

For DNA preparation 5×10^6 cells were resuspended in 2.5 ml extraction buffer (10 mM Tris-HCl, 400 mM NaCl, 2 mM EDTA, pH 8.2), with 0.5% SDS and 150 μg/ml proteinase K and incubated for 1 hour at 56°C. Subsequently, 0.8 ml saturated NaCl solution were added. After vigorous shaking and centrifugation, the DNA in the supernatant was precipitated with isopropanol, resuspended in TE, dispensed into aliquots, and stored at −80°C.

For RNA preparation, 2×10^5 cells were resuspended in lysis buffer (4.5 M GuSCN, 20 mM EDTA, 1.2% Triton X-100, 44 mM Tris-HCl, pH 6.4) to a final volume of 1 ml. Nucleic acids were bound to activated silica

TABLE I. Primer Pairs and Probes for VZV PCR and NASBA*

Sequence	Product length	Length of competitive standard	Sensitivity of assay
(ORF-14 PCR)			
p1: 5′-ACGTTCCCTCCGTGGTAGCATCC-3′			
p2: 5′-GGCGGCCTTTAAGCAGCAACAA-3′	338 bp	283 bp	80 copies/μg genomic DNA
probe: 5′-GGCGTATAAGCGACTTCCACC-3′			
(ORF-29 PCR)			
p1: 5′-TGCCGGAGCTGGTATTACCTTA-3′			
p2: 5′-ACAATGCCGTGACCACCAAGT-3′	266 bp	190 bp	50 copies/μg genomic DNA
probe: 5′-TTTCTGGCTCTAATCCAAGG-3′			
(ORF-63 PCR)			
p1: 5′-GTTTTGTACTCCGGGTTG-3′			
p2: 5′-TTACATCCGATGGCGTAG-3′	386 bp	350 bp	80 copies/μg genomic DNA
probe: 5′-AGACGCAGTGCTTACGCGCTACTT-3′			
(ORF-63 PCR, nested)			
p1: 5′-GCTCGTTGAGGACATGAACCGTGTT-3′			
p2: 5′-CATCGTCGCTATCGTCTTCACAC-3′	326 bp		10 copies/μg genomic DNA
probe: 5′-AGACGCAGTGCTTACGCGCTACTT-3′			
(ORF-63 NASBA)			
p1: 5′-AATTCTAATACGACTCACTATAGGGAAAGGAACATTCGGCGCCTCAA-3′			
p2: 5′-GCGCATATGCCTCGACTTTTATCT-3′	183 b		80 copies/μg nucleic acid
probe: 5′-TTAGCGTGCTGGGAGGAATTGTT-3′			
(ORF-68 NASBA)			
p1: 5′-AATTCTAATACGACTCACTATAGGGAAAATGATCTACTGTGGA-3′	187 b		65 copies/μg nucleic acid
p2: 5′-CACCGCATATTGTCTGG-3′			
probe: 5′-GAGAGTTTGTCGGGATTAT-3′			

*All given positions are according to the sequence of the strain Dumas [Davison and Scott, 1986]. Underlined nucleotides represent T7 promoter sequence.

(Sigma, size selected suspension in 10 mM HCl). After a series of rinses with 70% ethanol and acetone, the pellet was dried and nucleic acids were eluted in 100 μl H_2O at 56°C, dispensed into aliquots, and stored at −80°C. RNA isolation was evaluated by performing the U1A-NASBA (Organon Teknika, Boxtel, The Netherlands) combined with the ELGA (enzyme linked gel assay) detection system (van der Vliet et al., 1993).

Polymerase Chain Reaction (PCR)

PCR was done under standard reaction conditions [Saiki et al., 1988]. Optimal results were achieved using a $MgCl_2$ concentration of 1.4 mM (ORF 14), 1.0 mM (ORF 29), and 1.5 mM (ORF 63), respectively. The DNA was amplified in a total reaction volume of 50 μl (ORF-29 PCR) or 100 μl (ORF-14 PCR, ORF-63 PCR) consisting of the appropriate amount of $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 0.1% NP 40, 10 μg/100 μl BSA, 200 μM of each dNTP (Pharmacia, Sweden), 40 pmol of each primer and 2.5 U Taq polymerase (MBI Fermentas, Lithuania). The sample was added in a volume of 10 μl, containing 1 μg genomic DNA. Amplification was performed in a Trio Thermocycler (Biometra, Germany) as follows: [initial denaturation] 2 min at 94°C, [1] 1 min at 94°C, [2] 1.40 min at 54°C (ORF-14 PCR), 1.30 min at 64°C (ORF-29 PCR) or 1.30 at 49°C (ORF-63 PCR), [3] 1.45 min at 72°C. Steps [1] to [3] were repeated 40 times (ORF-14 PCR), 35 times (ORF-29 PCR) and 38 times (ORF-63 PCR), respectively. Nested PCR for ORF 63 was done by transferring 10 μl aliquots after 26 cycles into 90 μl reaction mixture containing inner primers and performing 26 cycles with 1 min at 94°C, 1.12 min at 54°C and 1.30 min at 72°C.

Quantification of PCR Products

Quantitative competitive PCR assays were established to determine the amount of VZV DNA in PBMC from patients with varicella and zoster. Briefly, internal competitive fragments were constructed for ORF-14 PCR by hybrid primer technique [Celi et al., 1993], for ORF-29 PCR by low-stringency primer annealing [Förster, 1994] and for the ORF-63 PCR by base deletion [Gilliland et al., 1990]. These modifications enabled us to distinguish amplified wild-type (wt) DNA and competitive standard (cs) DNA by size after electrophoresis through 9% polyacrylamid gels followed by ethidium bromide staining. Efficiency of coamplification was shown using dilution series of linearized plasmids containing wt and cs DNA sequences. Quantification was done by titration of a constant amount of sample DNA with different amounts of cs DNA. The number of copies of viral DNA in 1 μg genomic DNA was determined from the point where the amount of cs DNA and wt DNA were equivalent. Quantitative analysis was omitted when viral DNA could be detected only by nested PCR, which does not guarantee exponential amplification. However, the copy number of VZV DNA per μg genomic DNA in these samples must be in the range of 10 to 80 due to the sensitivity of the standard and nested PCR assays.

NASBA

RNA amplification was performed in a 20 μl volume, containing 40 mM Tris (pH 8.5), 12 mM $MgCl_2$, 70 mM KCl, 5 mM DTT, 2 mM of each NTP (Pharmacia), 1 mM of each dNTP, 1.5 M Sorbitol, 0.08 U RNase H (Phar-

macia), 32 U T7 RNA polymerase (Pharmacia), 6.4 U avian myeloblastosis virus reverse transcriptase (Seikagaku, U.S.A.), 0.1 μg/μl BSA (Boehringer Mannheim, Mannheim, Germany), 0.2 μM primer 1, 2, and 5 μl of isolated nucleic acids. The primer sequences were located either in the ORF 63 or in the ORF 68 of the VZV genome. Amplification was done as described by Kievits et al. [1991]. Briefly, after having added the sample RNA to a premix containing buffer ingredients and primers, the reaction mixture was incubated for 5 min at 65°C to uncoil secondary RNA structures. The samples were cooled to 41°C and the enzyme mix was added. Isothermal RNA amplification was performed by incubation at 41°C for 90 min.

Detection of Amplified DNA and RNA

PCR and NASBA products were analyzed by agarose gel electrophoresis (1.8%) followed by ethidium bromide staining and Southern/Northern blotting. Hybridization was performed either with ^{32}P-labeled oligonucleotide probes or with digoxigenin-labeled doublestranded, denaturated DNA probes/digoxigenin-labeled in vitro RNA (DIG Labeling and Detection Kit, Boehringer Mannheim).

Immunofluorescence

For the detection of the viral glycoprotein gE $2 \cdot 10^6$, PBMC were fixed in 4% paraformaldehyde/PBS for 30 min. The cells were incubated successively with FITC-conjugated anti-CD4/CD8 antibodies (Dianova, Hamburg, Germany), with monoclonal anti-gE (clone VL8, mouse IgG1) [Nikkels et al., 1995] and LRSC-labeled goat-anti-mouse IgG (Dianova). Excess antibody was removed by washing with PBS. Cells were resuspended in 1% paraformaldehyde/PBS and visualized by laser scanning confocal microscopy using the Leitz DM IRB microscope connected to the Leica True Confocal Scanner (Leica, Heidelberg, Germany).

RESULTS

Each specimen was examined for viral nucleic acids using five different assays: three PCR assays for the detection of ORF 14, ORF 29, and ORF 63 and two NASBA assays for the detection of RNA transcribed from genes 63 and 68, respectively. The sensitivity for each PCR and NASBA reaction was established by amplification of serial dilutions of VZV-specific linearized plasmid DNA and in vitro RNA, respectively. The sensitivity of each reaction is given in Table I. Specificity was demonstrated by amplifying nucleic acids extracted from cell cultures. No specific amplification was observed with nucleic acids isolated from either uninfected or HSV-1 infected fibroblasts. Controls prepared from cells infected with VZV strains Ellen, Oka, or Webster gave positive results in each PCR and NASBA. Each assay was carried out in duplicate. If the results were not identical, the assay was repeated twice. A sample was considered positive if VZV-specific sequences were detected in at least two of the four attempts.

Table II summarizes PCR and NASBA analysis of PBMC from varicella patients. VZV-specific nucleic acids were detected in 10 of 21 blood samples from subjects with varicella. Samples taken from patients treated with acyclovir as well as samples taken more than 8 days after the onset of rash scored negative. VZV DNA was found either using the standard PCR assays or exclusively with the nested ORF-63-PCR, concurrent to the sensitivity of each assay. Additionally, quantitative competitive PCR (QCPCR) was undertaken for the detection of ORF-14 DNA, ORF-29 DNA , and ORF-63 DNA on four samples (subjects no. 2, 5, 6, and 11). 200 to 5,000 copies of VZV DNA per μg genomic DNA, equivalent to 150,000 PBMC, were found (Fig. 2). It is important to note that for each sample the results obtained in the three PCR assays were in a narrow range (subject 2: 200 – 500 copies; subject 5: 3,500 – 5,000 copies; subject 6: 1,000 – 2,000 copies; subject 11: 3,000 – 5,000 copies). VZV RNA transcribed from both gene 63 and gene 68 were detected in 6 out of 17 samples (Fig. 3). In a further sample viral RNA was found exclusively in the ORF-63 NASBA. The NASBA results corresponded to those obtained by PCR (Table II), indicating viral transcriptional activity in VZV infected PBMC.

The amount of cells and the type of PBMC infected during the viremic phase were determined by doublestaining PBMC from subjects no. 3 and 6 with the anti-gE-antibody and anti-CD4/CD8-antibodies. Immunofluorescence was visualized by confocal laser scanning microscopy. The frequency of PBMC expressing the glycoprotein gE ranged from 1 in 10,000 to 100,000 cells. As revealed by CD4/CD8-expression and analysis of cell morphology, about two-thirds of the infected PBMC were T lymphocytes, the remaining third consisted of both B lymphocytes and monocytes (Fig. 4).

PBMC from 71 immunocompetent zoster patients were investigated using PCR and NASBA. Samples were collected between day 1 and day 12 after the onset of pain and/or rash. As shown in Table III, we were able to detect VZV-specific nucleic acids in 11 of 71 samples (16%). None of the samples taken later than 6 days after the onset of rash or drawn from acyclovir-treated patients scored positive. It was concluded, therefore, that at least sometimes a viremic phase can be observed in immunocompetent subjects with acute zoster in the absence of antiviral therapy. As shown in patients with varicella, the PBMC of zoster patients are also productively infected because RNA transcribed from the late VZV gene 68 was detected . However, the viral load appeared to be lower in zoster patients than in those with varicella. Apart from two exceptions, nested PCR that was eight times more sensitive than standard PCR (Table I) had to be applied to detect VZV DNA.

PBMC from 16 patients suffering from postherpetic neuralgia were analyzed. Pain in these patients had persisted between 2 months and 7 years. Two of them were being treated with acyclovir at the time samples were taken. VZV DNA or VZV RNA were not detected in any of the samples.

TABLE II. Detection of VZV DNA and RNA in Patients With Varicella

Subject no.	Days since onset of rash	IgM/IgG	Detection of VZV-specific nucleic acids					
			PCR				NASBA	
			ORF-14	ORF-29	ORF-63 standard	ORF-63 nested	ORF-63	ORF-68
1	1	+/–	–	+	–	+	+	+
2	1	–/–	+	+	+	n.d.	+	+
3	2	+/–	–	–	–	+	+	+
4	3	+/–	n.d.	n.d.	–	+	n.d.	n.d.
5	3	+/–	+	+	+	n.d.	+	+
6	3	+/–	+	+	+	n.d.	+	+
7[a]	4	+/+	–	–	–	–	–	–
8	4	+/+	–	–	–	–	–	–
9[a]	4	+/+	–	–	–	–	–	–
10	5	+/+	+	+	+	n.d.	+	–
11	5	+/+	+	+	+	n.d.	+	+
12	7	+/+	n.d.	n.d.	+	n.d.	n.d.	n.d.
13	8	+/+	n.d.	n.d.	–	+	n.d.	n.d.
14	8	+/+	–	–	–	–	–	–
15	8	+/+	–	–	–	–	–	–
16	9	+/–	–	–	–	–	–	–
17	9	+/+	–	–	–	–	–	–
18	12	+/+	n.d.	n.d.	–	–	n.d.	n.d.
19	12	+/+	–	–	–	–	–	–
20	12	+/+	–	–	–	–	–	–
21	?	+/–	–	–	–	–	–	–

[a]Subjects treated with acyclovir.
n.d. = not done.

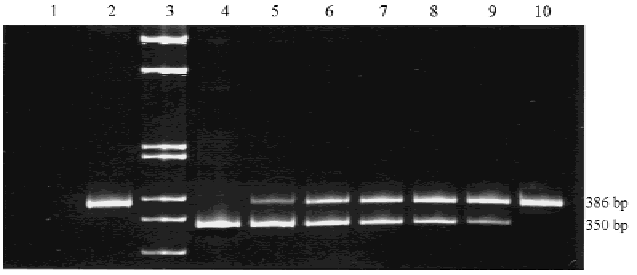

Fig. 2. Quantitative PCR for the detection of VZV ORF-63 DNA after polyacrylamide gel electrophoresis and ethidium bromide staining. DNA (1 μg) extracted from PBMC of subject 6 was mixed with different amounts of competitive standard (cs) DNA. Numbers at the right side indicate the size of the amplified fragments in base pairs (bp). Lane 1, no DNA (negative control); lane 2, PBMC DNA from subject 6; lane 3, molecular size marker; lanes 4 to 10, PBMC DNA from subject 6 mixed with 5,000, 4,000, 3,000, 2,000, 1,000, 500, 100 copies of cs DNA, respectively. The point of equivalence was obtained at about 2,000 copies of input cs DNA.

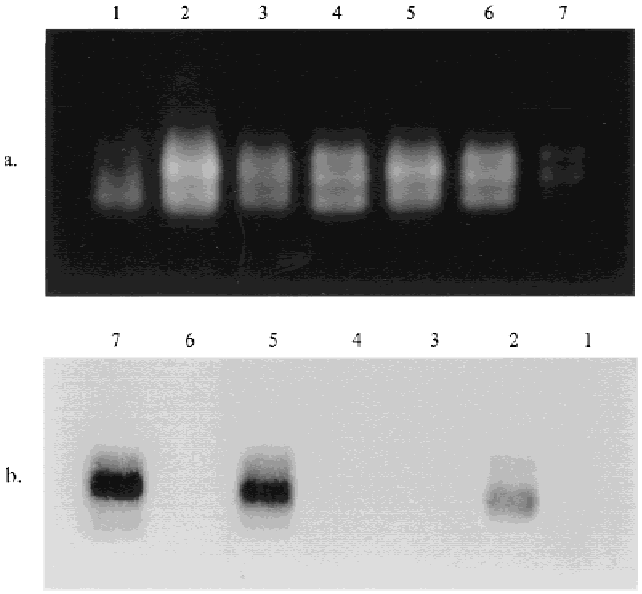

Fig. 3. VZV RNA detected by ORF-63 NASBA. NASBA products after agarose gel electrophoresis and ethidium bromide staining **(a)** and after Northern blotting and hybridization with a DIG-labeled probe **(b).** Lane 1, no RNA (negative control); lanes 2 to 6, RNA extracted from PBMC of subjects no. 5, 7, 8, 1, and 21, respectively; lane 7, RNA isolated from VZV-infected fibroblasts.

To investigate the occurrence of subclinical VZV reactivations blood samples were taken from immunocompetent as well as immunocompromised individuals. VZV DNA was found in 3 of 99 immunocompetent subjects and in 2 of 50 immunocompromised subjects who were VZV antibody positive (IgG positive, IgM negative). However, viral DNA was detected exclusively by nested ORF-63 PCR and VZV RNA was not found in any of these samples. VZV-specific nucleic acids were not detected in any of the 14 PBMC samples from seronegative donors.

DISCUSSION

After primary infection, VZV is disseminated to epithelial cells by PBMC-associated viremia [Asano et al., 1985; Koropchak et al., 1991]. However, it has been difficult to characterize the viremic phase in detail. VZV-infected PBMC have been found in immunocompromised zoster patients [Feldmann et al., 1977]. Whether viremia contributes to the symptoms seen in patients with postherpetic neuralgia, the most common complication following herpes zoster, has not yet been

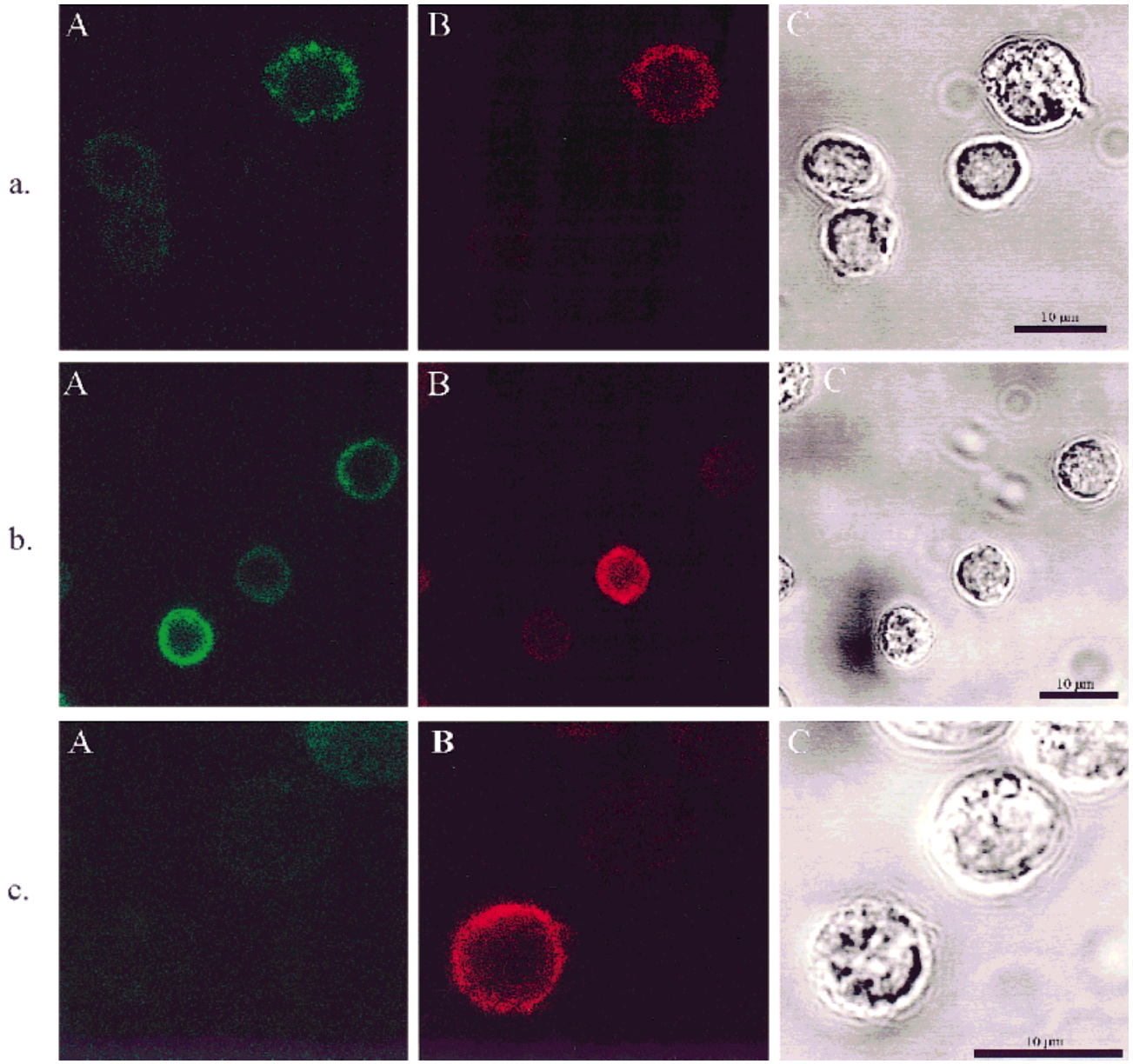

Fig. 4. Detection of VZV glycoprotein gE on the surface of PBMC from varicella patients. Immunofluorescence was visualized by confocal laser scanning microscopy. Green fluorescence after staining of PBMC with FITC-conjugated anti-CD4/CD8 antibodies **(A).** Red fluorescence after staining PBMC with anti-gE primary and LRSC-labeled secondary antibodies **(B).** Transmission images **(C).** Expression of gE was revealed on CD4/CD8 positive T lymphocytes **(a)** and **(b)** as well as on CD4/CD8 negative PBMC **(c).**

resolved. Thus, our study aimed to characterize viremia in varicella and immunocompetent zoster patients and to elucidate whether postherpetic neuralgia is associated with VZV infection of PBMC. Furthermore, we looked for subclinical reactivation in asymptomatic individuals. Choosing a methodological approach that covered the detection of viral DNA, RNA, and protein in blood cells of these subjects, it was possible to distinguish between a mere infection and a viral replication within cells.

Sequences coding for immediate early, early, and late proteins in different regions of the viral genome were chosen to look for VZV DNA in PBMC by PCR. With ORF-14, ORF-29, and/or ORF-63, viral DNA was found in 10 out of 21 (48%) blood samples taken from varicella patients and 10 out of 71 (14%) immunocompetent zoster patients. However, all specimens that gave positive results were drawn during the first week after the onset of the rash. Viral DNA in PBMC of varicella patients was detectable more often using standard PCRs than in those of zoster patients where nested PCR had to be performed, with two exceptions. Therefore, the viral load seems to be lower in the case of zoster than varicella. When PBMC were examined from 16 postherpetic neuralgia patients with postherpetic neuralgia by PCR all were negative. But VZV

TABLE III. Samples From zoster patients who Gave Positive Results by PCR and/or NASBA

Subject no.	Days since onset of symptoms	IgM/IgG	Detection of VZV-specific nucleic acids					
			PCR				NASBA	
			ORF-14	ORF-29	ORF-63 standard	ORF-63 nested	ORF-63	ORF-68
1	1	–/+	–	–	–	+	–	–
2	2	–/+	–	–	–	+	–	–
3	2	–/+	–	–	–	+	+	–
4	2	+/+	–	–	–	+	+	+
5	3	–/+	–	+	–	+	+	–
6	3	–/+	+	+	+	n.d.	–	–
7	3	–/+	–	–	–	+	+	+
8	3	–/+	–	–	–	+	+	+
9	5	–/+	–	–	–	+	+	+
10	6	–/+	–	–	–	–	+	+
11	6	–/+	–	–	–	+	–	–

DNA was detected in 3 of 99 immunocompetent and 2 of 50 immunocompromised individuals without any signs of active VZV infection. Using quantitative competitive PCR, the amount of VZV DNA in PBMC was determined in four patients with acute varicella. The copy number of viral DNA varied among 200 and 5,000 copies per μg genomic DNA in the four samples. Since the amount of viral DNA was similar when the different QCPCR assays were applied on the same sample, it is concluded that the majority of infected cells contain the complete VZV genome. However, it was not possible to draw any conclusion on the actual number of PBMC infected by VZV,because one cell can contain several copies of the genome.

Next we aimed to investigate viral transcriptional activity in VZV infected PBMC. NASBA has been shown to be a fast, sensitive, and reliable method for RNA amplification [Bruisten et al., 1993; Smits et al., 1995]. The main advantage of NASBA is that selective RNA amplification is not influenced by a DNA background. Two assays were developed to detect viral RNA transcribed from an immediate early (ORF 63) as well as from a late gene (ORF 68). The first is found in latent and productive infected cells, whereas transcription of gene 68 indicates viral replication. All samples were examined by these two NASBA assays. VZV RNA was found in PBMC from varicella and zoster patients, but not in those with postherpetic neuralgia and asymptomatic individuals. With one exception, all samples that exhibited viral transcriptional activity were also positive by PCR. Since transcripts from the immediate early gene 63 were detected as well as from the late gene 68, it is suggested that VZV indeed replicates in PBMC.

To determine the number and the type of these blood cells, immunofluorescence was used since VZV infected cells carry viral glycoproteins on their surface (Montalvo et al., 1985). It is known that infected PBMC are fragile and easily destroyed by fluorescence-activated cell sorting [Koropchak et al., 1989]. Therefore, PBMC were doublestained with viral and T-lymphocyte markers. Additionally, visualizing cells with confocal laser scanning microscopy allowed the analysis of cell morphology. It was found that only 0.01 to 0.001% of PBMC carry the virus. The results are in accordance with observations of Koropchak et al. [1989], who found about the same frequency of infected cells using in situ hybridization with a labeled DNA probe. The majority of VZV-infected PBMC were T lymphocytes as shown by expression of CD4 or CD8. After analyzing the cell morphology of the remaining cells it is suggested that B cells and monocytes also expressed gE on their surface. Therefore, our studies reveal that several PBMC subpopulations are susceptible to VZV infection during viremia.

In conclusion, the data suggest the existence of productively infected PBMC in varicella as well as in zoster patients. Viremia seems to be transient because viral sequences were only detectable in the first few days after the onset of rash. Antiviral therapy appears to terminate the viremic phase, since VZV-specific nucleic acid was not detected in patients being treated with acyclovir. The detection of VZV DNA and RNA in immunocompetent zoster patients offers an explanation for the observation that even in case of segmentally restricted zoster one or few lesions can be found outside the involved dermatome.

Vafai et al. [1988] found VZV DNA and proteins in PBMC from patients with postherpetic neuralgia. It was concluded that persisting viral replication may be responsible for the continuous pain. We were unable to demonstrate a correlation between postherpetic neuralgia and the incidence of VZV DNA in PBMC. Therefore, the data contradict the previous study of Vafai et al. [1988], but favors the concept of postherpetic neuralgia caused by neuronal cell damage during acute zoster as proposed by Kost and Straus [1996].

Hope-Simpson [1965] previously proposed that subclinical reactivation of latent VZV contributes to booster immunity against VZV. Asymptomatic VZV viremia was reported in immunocompromised subjects [Ljungman et al., 1986; Wilson et al., 1992]. Devlin et al. [1992] demonstrated viral DNA in PBMC from elderly adults. In accordance with these results, VZV DNA was detected in 5 of 149 individuals, none of whom had a history of zoster. Viral nucleic acids were exclusively found with nested ORF-63 PCR implying a low viral load. Since we were unable to detect viral

transcriptional activity in these samples, productive infection of PBMC is uncertain. But the existence of latent infected PBMC in these persons seems unlikely, because blood samples taken several weeks later were negative. The three immunocompetent subjects with subclinical reactivations were >60 years old, suggesting a correlation between age and the detection of VZV DNA. However, since 78 samples were collected from adults >60 and only 21 samples from adults <60, this finding may be coincidental. The results confirm the observations by Devlin et al. [1992] that subclinical reactivations occur in both immunocompromised subjects and healthy individuals.

In summary, the above study allowed further insight into the occurrence of viremia in different stages of VZV infection. Productive infection of PBMC was demonstrated in patients with acute varicella and zoster. With regard to the etiology of postherpetic neuralgia, it is proposed that active viral replication does not contribute to this condition. The results indicate that subclinical reactivations occur in immunocompromised and in immunocompetent individuals and booster cellular immunity. This may be one reason why clinical VZV reactivation in contrast to HSV reactivation is a rare event, at least in immunocompetent individuals.

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